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(54) Title: AMYLIN ANTAGONISTS AND AGONISTS

(57) Abstract

The invention features amylin analogs which behave as amylin antagonists and agonists. The invention also features the use of the amylin antagonist for the treatment of Type II diabetes mellitus, and the use of the amylin agonists for the treatment of both Type I diabetes mellitus and hypercalcemia. The invention also features the use of amylin antagonists and agonists for the control of food intake.

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# AMYLIN ANTAGONISTS AND AGONISTS

## Background of the Invention

This invention relates to specific amylin analogs which behave as amylin antagonists and agonists, and to their use in the treatment of diabetes mellitus, and hypercalcemia, and the control of food intake.

Amylin, also known as diabetes associated polypeptide (Cooper et al., Proc. Natl. Acad. Sci. USA, 85:7763-7766 (1988)) or islet/insulinoma amyloid polypeptide (Westermark et al., Proc. Natl. Acad. Sci. USA, 84:3881-3885 (1987)), is a 37-residue polypeptide amide isolated originally from the amyloid-rich pancreas of insulinoma and noninsulin-dependent diabetic (NIDD) patients. It has subsequently been isolated from the normal pancreas of rat (Asai et al., Biochem. Biophys. Res. Commun., 164:400-405 (1989)). CDNA cloning (Ferrier et al., J. Mol. Endocrinol., 3:R1-R4 (1989)) and immunocytochemical (Lukinius et al., Diabetologia, 32:240-244 (1989)) studies have demonstrated that amylin is synthesized in the islet cells and stored in the islet secretory granules along with insulin. It is cosecreted with insulin (Kanatsuka et al., FEBS Lett., 259:199-201 (1989)). Low quantities of amylin have also been detected in the stomach, intestine, lung and dorsal root ganglion (Asai et al., Biochem. Biophys. Res. Commun., 169:788-795 (1990)); and Ferrier et al., supra).

Biological investigations that followed the isolation of amylin have shown that amylin inhibits basal and insulin-stimulated glucose uptake as well as glycogen synthesis by soleus muscles (Leighton et al., Nature, 335:632-635 (1988)). This peripheral insulin resistance by amylin has also been demonstrated in vivo by euglycemic glucose clamp studies with dogs (Sowa et al.,

Diabetologia, 33:118-120 (1990)) and rats (Molina et al., Diabetes, 39:260-265 (1990)). Furthermore, these investigations in rats showed that amylin attenuated the inhibition of hepatic glucose output by insulin (Molina et al., supra). Based on these observations and the finding that amylin inhibits basal insulin secretion (Ohsawa et al., Biochem. Biophys. Res. Commun., 160:961-967 (1989)), it has been suggested that amylin might play a role in glucose metabolism and the pathophysiology of noninsulin-dependent diabetes mellitus (NIDDM), commonly known as Type II diabetes mellitus.

Diabetes mellitus is a metabolic disease characterized by chronic hyperglycemica, i.e., elevated blood sugar levels. This disease affects a significant percentage of the population. There are two major categories of diabetes mellitus, commonly referred to as Type I and Type II. In patients with Type I diabetes mellitus, there is a loss of active $\beta$-cells in the islets of Langerhans in the pancreas, resulting in low levels of both insulin and amylin. Cooper, Medical Hypothesis, 26:284-288 (1991). Patients with Type I diabetes mellitus who are treated with insulin frequently have a tendency to develop hypoglycemia as a side effect. In patients with Type II diabetes mellitus, there are elevated levels of amylin. Patients with type II diabetes mellitus display varying resistance to the normal biological effects of insulin. Increased levels of amylin, known as hyperamylinemia, have been implicated in causing insulin resistance in a number of model systems, including genetically obese LA/N-cp rats (Huang et al., Hypertension, 19:i-101 - i-109 (1992)), genetically obese diabetic yellow mice (Gill et al., Life Sci, 48:703-718 (1991)), dexamethasone induced diabetic rats (Jamal et al., J. Endocrin., 126:425-429 (1990)), streptozocin induced diabetic rats (Inoue et al.,

Diabetes, 41:723-727 (1992)), and ventromedial hypothalamic lesioned rats and Zucker rats (Tokuyama et al., Endocrinology, 128:2739-2744 (1991)).

Other studies have shown that amylin, like calcitonin, can exhibit serum calcium-lowering effects in rats in vivo as well as in cell culture systems (Datta et al., Biochem. Biophys. Res. Commun., 162:876-881 (1989)). Amylin has also been shown to act as an anorectic agent. Balasubramaniam et al., Peptides, 12:919-924 (1991).

## Summary of the Invention

In general, the invention features amylin analogs which behave as amylin antagonists and agonists.

In one aspect, the invention features amylin analogs which are linear analogs of biologically active amylin having the following amino acid formula:

$$R_1$$
$$\backslash$$
$$R_2\text{-}X\text{-}A^8\text{-}A^9\text{-}A^{10}\text{-}A^{11}\text{-}A^{12}\text{-}A^{13}\text{-}A^{14}\text{-}A^{15}\text{-}A^{16}\text{-}A^{17}\text{-}A^{18}\text{-}A^{19}\text{-}A\text{-}^{20}\text{-}A^{21}\text{-}A^{22}\text{-}A^{23}\text{-}Y\text{-}Z$$

wherein:

X is a chain of 0-5 amino acids, inclusive, the N-terminal one of which is bonded to $R_1$ and $R_2$;

Y is a chain of 0-4 amino acids, inclusive, the C-terminal one of which is bonded to Z;

Each of $R_1$, and $R_2$, independently, is H, $C_1$-$C_{12}$ alkyl (e.g., methyl), $C_6$-$C_{18}$ aryl (e.g., phenyl, naphthaleneacetyl), $C_1$-$C_{12}$ acyl (e.g., formyl, acetyl, and myristoyl), $C_7$-$C_{18}$ aralkyl (e.g., benzyl), or $C_7$-$C_{18}$ alkaryl (e.g., *p*-methylphenyl);

$A^8$ is Ala, Nal, Thi, Phe, Bth, Pcp, N-Me-Thr, Aib, or Anb;

$A^9$ is Thr, Ala, Anb, Aib, Ser, N-Me-Ser, or N-Me-Thr;

$A^{10}$ is Gln, Ala, Asn, N-Me-Gln, Gly, Nva, Aib, or Anb;

$A^{11}$ is Arg, homo-Arg, diethyl-homo-Arg, Lys-$\epsilon$-NH-R (where R is H, a branched or straight chain $C_1$-$C_{10}$ alkyl group, or an aryl group), Orn, or Lys;

$A^{12}$ is Leu, Ile, Val, Aib, Anb, or N-Me-Leu;

$A^{13}$ is Ala, Nal, Thi, Phe, Bth, Pcp, N-Me-Thr, Aib, or Anb;

$A^{14}$ is Asn, Ala, Gln, Gly, N-Me-Asn, Nva, Aib, or Anb;

$A^{15}$ is Phe, or any aromatic amino acid with or without substituents;

$A^{16}$ is Leu, Ile, Val, Aib, Anb, or N-Me-Leu;

$A^{17}$ is Val, Ile, Aib, Anb, or N-Me-Val;

$A^{18}$ is His, Thr, 3-Me-His, 1-Me-His, $\beta$-pyrozolylalanine, N-Me-His, Arg, homo-Arg, diethyl-homo-Arg, Lys-$\epsilon$-NH-R (where R is H, a branched or straight chain $C_1$-$C_{10}$ alkyl group, or an aryl group), Ala, Aib, Anb, or Orn;

$A^{19}$ is Ser, Thr, N-Me-Ser, N-Me-Thr, Aib, Anb, or Ala;

$A^{20}$ is Ser, Thr, N-Me-Ser, N-Me-Thr, Aib, Anb, or Ala;

$A^{21}$ is Asn, Ala, Gln, Gly, N-Me-Asn, Aib, Anb, or Nva;

$A^{22}$ is Asn, Ala, Gln, Gly, N-Me-Asn, Aib, Anb, or Nva;

$A^{23}$ is Phe, any aromatic amino acid with or without substituents, Leu, Ile, Val, Aib, Anb, Ala, or N-Me-Leu; and

Z is $NHR_3$ or $OR_3$; wherein $R_3$ is H, $C_1$-$C_{12}$ alkyl, $C_7$-$C_{10}$ phenylalkyl, $C_3$-$C_{20}$ alkenyl, $C_3$-$C_{20}$ alkinyl, phenyl, or naphthyl.

In preferred embodiments, the analogs are antagonists. In a highly preferred embodiment, the amylin antagonist corresponds to the N-α acetyl derivative of amino acids 8 through 23 of human amylin with an amidated carboxy at the C-terminus, referred to herein as N-α-ac-human amylin (8-23)-$NH_2$, having the following formula:

N-α-Ac-Ala-Thr-Gln-Arg-Leu-Ala-Asn-Phe-Leu-Val-His-Ser-Ser-Asn-Asn-Phe-$NH_2$ (SEQ ID NO:1)

In another preferred embodiment, the amylin antagonist has the following formula:

N-α-Ac-Ala-Thr-Gln-Arg-Leu-Ala-Asn-Phe-Leu-Val-Arg-Ser-Ser-Asn-Asn-Leu-$NH_2$. (SEQ ID NO:2)

In another aspect, the invention features amylin analogs which are linear analogs of biologically active amylin having the following amino acid formula:

$$R_2-X-A^1-A^2-A^3-A^4-A^5-A^6-A^7-A^8-A^9-A^{10}-A^{11}-A^{12}-A^{13}-A^{14}-A^{15}-A^{16}-A^{17}-A^{18}-A^{19}-A^{20}-A^{21}-A^{22}-A^{23}-Y-Z$$

wherein

X is a chain of 0-5 amino acids, inclusive, the N-terminal one of which is bonded to $R_1$ and $R_2$;

Y is a chain of 0-4 amino acids, inclusive, the C-terminal one of which is bonded to Z;

Each of $R_1$, and $R_2$, independently, is H, $C_1$-$C_{12}$ alkyl, $C_6$-$C_{18}$ aryl, $C_1$-$C_{12}$ alyl, $C_7$-$C_{18}$ aralkyl, or $C_7$-$C_{18}$ alkaryl;

$A^1$ is Lys, Arg, homo-Arg, diethyl-homo-Arg, Lys-ε-NH-R (where R is H, a branched or straight chain $C_1$-$C_{10}$ alkyl group, or an aryl group), or Orn;

$A^2$ is Cys, or Anb;

$A^3$ is Asn, Ala, Gln, Gly, N-Me-Asn, Aib, Anb, or Nva;

$A^4$ is Thr, Ser, N-Me-Ser, N-Me-Thr, Ala, Aib, or Anb;

$A^5$ is Ala, Nal, Thi, Phe, Bth, Pcp, N-Me-Ala, Aib, or Anb;

$A^6$ is Thr, Ser, N-Me-Ser, N-Me-Thr, Ala, Aib, or Anb;

$A^7$ is Cys, or Anb;

$A^8$ is Ala, Nal, Thi, Phe, Bth, Pcp, N-Me-Ala, Aib, or Anb;

$A^9$ is Thr, Ser, N-Me-Ser, N-Me-Thr, Ala, Aib, or Anb;

$A^{10}$ is Gln, Ala, Asn, N-Me-Gln, Gly, Nva, Aib, or Anb;

$A^{11}$ is Arg, homo-Arg, diethyl-homo-Arg, Lys-$\epsilon$-NH-R (where R is H, a branched or straight chain $C_1$-$C_{10}$ alkyl group, or an aryl group), or Orn;

$A^{12}$ is Leu, Ile, Val, Aib, Anb, or N-Me-Leu;

$A^{13}$ is Ala, Nal, Thi, Phe, Bth, Pcp, N-Me-Ala, Aib, or Anb;

$A^{14}$ is Asn, Ala, Gln, Gly, N-Me-Asn, Aib, Anb, or Nva;

$A^{15}$ is Phe, or any aromatic amino acid with or without substituents;

$A^{16}$ is Leu, Ile, Val, Aib, Anb, or N-Me-Leu;

$A^{17}$ is Val, Ile, Aib, Anb, or N-Me-Val;

$A^{18}$ is His, Thr, 3-Me-His, 1-Me-His, $\beta$-pyrozolylalanine, N-Me-His, Arg, homo-Arg, diethyl-homo-Arg, Lys-$\epsilon$-NH-R (where R is H, a branched or straight chain $C_1$-$C_{10}$ alkyl group, or an aryl group), Orn, Ala, Aib, or Anb;

$A^{19}$ is Ser, Thr, N-Me-Ser, N-Me-Thr, Ala, Aib, or Anb;

$A^{20}$ is Ser, Thr, N-Me-Ser, N-Me-Thr, Ala, Aib, or Anb;

$A^{21}$ is Asn, Ala, Gln, Gly, N-Me-Asn, Aib, Anb, or Nva;

$A^{22}$ is Asn, Ala, Gln, Gly, N-Me-Asn, Aib, Anb, or Nva;

$A^{23}$ is Phe, any aromatic amino acid with or without substitutions, Leu, Ile, Val, Aib, Anb, Ala, or N-Me-Leu; and

Z is $NHR_3$ or $OR_3$; wherein $R_3$ is H, $C_1$-$C_{12}$ alkyl, $C_7$-$C_{10}$ phenylalkyl, $C_3$-$C_{20}$ alkenyl, $C_3$-$C_{20}$ alkinyl, phenyl, or naphthyl.

In one highly preferred embodiment, the amylin analog corresponds to amino acids 1 through 23 of human amylin with an amidated carboxy at the C-terminus, referred to herein as human amylin (1-23)-$NH_2$, having the following formula:

Lys-Cys-Asn-Thr-Ala-Thr-Cys-Ala-Thr-Gln-Arg-Leu-Ala-Asn-Phe-Leu-Val-His-Ser-Ser-Asn-Asn-Phe-$NH_2$. (SEQ ID NO:3)

In another highly preferred embodiment, the amylin analog corresponds to amino acids 1 through 23 of rat amylin, with an amidated carboxy at the C-terminus, referred to herein as rat amylin (1-23)-$NH_2$, having the following formula:

Lys-Cys-Asn-Thr-Ala-Thr-Cys-Ala-Thr-Gln-Arg-Leu-Ala-Asn-Phe-Leu-Val-Arg-Ser-Ser-Asn-Asn-Leu-$NH_2$. (SEQ ID NO:4)

In yet another highly preferred embodiment, the amylin analog corresponds to the derivative of amino acids 1 through 23 of rat amylin with α-amino normal butyric acid substitutions at positions 2 and 7, and an amidated

carboxy at the C-terminus, referred to herein as [$Anb^{2,7}$] rat amylin (1-23)-$NH_2$, having the following formula:

Lys-Anb-Asn-Thr-Ala-Thr-Anb-Ala-Thr-Gln-Arg-Leu-Ala-Asn-Phe-Leu-Val-Arg-Ser-Ser-Asn-Asn-Leu-$NH_2$. (SEQ ID NO:5)

In another aspect, the invention features a method of treating Type II diabetes mellitus in a human being by administering a therapeutic amount of an amylin antagonist of the invention. In a highly preferred method of treatment of Type II diabetes mellitus, N-α-ac-human amylin (8-23)-$NH_2$ is adminstered.

In another aspect, the invention features a method of treating Type I diabetes mellitus in a human being by administering a therapeutic amount of an amylin agonist of the invention in conjunction with a therapeutic amount of insulin.

In still another aspect, the invention features a method of treating hypercalcemia by administering a therapeutic amount of an amylin agonist of the invention.

The compounds of the invention exhibit a broad range of biological activities, including those related to glucose metabolism, calcium levels in the blood, and appetite. Amylin antagonists of the invention attenuate the inhibition by amylin of insulin-stimulated glucose uptake. As a result, the amylin antagonists of the invention act to reduce hyperglycemia resulting from elevated levels of amylin associated with Type II diabetes mellitus. Amylin agonists of the invention inhibit insulin stimulated glucose uptake, thereby tending to increase blood sugar levels. As a result, the amylin agonists of the invention are useful in reducing the hypoglycemia which frequently accompanies insulin treatment of Type I diabetes mellitus. Amylin agonists of the invention inhibit insulin stimulated glucose

uptake, thereby tending to increase blood sugar levels. As a result, the amylin agonists of the invention are useful in reducing the hypoglycemia which frequently accompanies insulin treatment of Type I diabetes mellitus. Amylin agonists of the invention also decrease serum calcium levels, and are therefore useful for treating hypercalcemia. In addition, amylin agonists exhibit an appetite suppressant effect, while amylin antagonists increase appetite. Amylin agonists and antagonists are therefore useful in controlling food intake. For example, amylin agonists are useful for treating problems of overweight.

Many of the compounds of the invention are especially advantageous because they are truncated versions of the natural amylin peptide. The shorter peptide not only facilitates easier synthesis and purification of the compounds, but also improves selectivity and reduces manufacturing procedures and expenses.

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof, and from the claims.

## Detailed Description

The drawings will first be briefly described.

### Drawings

Fig. 1 shows the comparison of the primary structures of human amylin (hAMYLIN) and rat amylin (rAMYLIN).

Fig. 2 shows the effect of human amylin, and N-α-ac-human amylin (8-23)-$NH_2$, separately and together, on glucose uptake in $C_2C_{12}$ muscle cells.

Fig. 3a and Fig. 3b show the in vivo effects of saline, rat amylin, N-α-ac-human amylin (8-23)-$NH_2$, and N-α-ac-human amylin (8-23)-$NH_2$ plus rat amylin on plasma

glucose levels, and plasma insulin levels, respectively, in Sprague Dawley rats.

Fig. 4 shows the in vitro effect of human amylin and human amylin (1-23)-$NH_2$, separately, on insulin stimulated glucose uptake in $C_2C_{12}$ muscle cells.

Fig. 5a and 5b show the in vivo effects of saline, rat amylin, human amylin (1-23)-$NH_2$, and human amylin (1-23)-$NH_2$ plus rat amylin on plasma glucose levels, and plasma insulin levels, respectively, in Sprague Dawley rats.

Fig. 6 shows the in vitro effects of rat amylin (1-23)-$NH_2$ and [$Anb^{2,7}$] rat amylin (1-23)-$NH_2$, separately, on insulin stimulated glucose uptake in $C_2C_{12}$ muscle cells.

Fig. 7a and 7b show the in vivo effects of saline, rat amylin, [$Anb^{2,7}$] rat amylin (1-23)-$NH_2$, and [$Anb^{2,7}$] rat amylin (1-23)-$NH_2$ plus rat amylin on plasma glucose levels, and plasma insulin levels, respectively, in Sprague Dawley rats.

## Structure

The sequences of naturally occuring human amylin ("hAmylin") and rat amylin ("rAmylin") are set forth in Fig. 1. Balasubramaniam et al., Peptides, 12:919-924 (1991). There is a high degree of sequence homology between amylin from these two species. It is believed that in naturally occuring hAmylin and rAmylin, the cysteine residues at positions 2 and 7, present in both species, form an internal disulfide bond, resulting in a cyclic structure.

The amylin analogs of the invention are based upon the biologically active subfragments comprising amino acids 8-23 of hAmylin and rAmylin and derivatives thereof; and upon the biologically active subfragments comprising amino acids 1-23 of hAmylin and rAmylin and

derivatives thereof. In the amylin analog formulas set forth herein, the symbols $A^X$ and the like; and Ser, Leu and the like, as found in a peptide sequence herein, stand for amino acid residues. When an amino acid residue is optically active, it is the L-form configuration that is intended unless the D-form is expressly designated. All peptide sequences mentioned herein are written according to the usual convention whereby the N-terminal amino acid is on the left and the C-terminal amino acid is on the right. A short line between two amino acid residues indicates a peptide bond. An -OR or an -NHR substituent on the carboxy terminal end of a peptide replaces the -OH on the carboxy terminal amino acid residue, yielding -NH-CH(R)-COOR, and -NH-CH(R)-CONHR as the C-terminal residues, respectively. When the carboxy terminal substituent is $-NH_2$, the peptide is in the amidated carboxy form.

As set forth above and for convenience in describing this invention, the conventional and nonconventional abbreviations for the various amino acids are used. They are familiar to those skilled in the art; but for clarity are listed below.

Asp = D = Aspartic Acid
Ala = A = Alanine
Arg = R = Arginine
Asn = N = Asparagine
Cys = C = Cysteine
Gly = G = Glycine
Glu = E = Glutamic Acid
Gln = Q = Glutamine
His = H = Histidine
Ile = I = Isoleucine
Leu = L = Leucine
Lys = K = Lysine

Met = M = Methionine
Phe = F = Phenylalanine
Pro = P = Proline
Ser = S = Serine
Thr = T = Threonine
Trp = W = Tryptophan
Tyr = Y = Tyrosine
Val = V = Valine
Orn = Ornithine
Nal = 2-napthylalanine
Nva = norvaline
Thi = 2-thienylalanine
Pcp = 4-chlorophenylalanine
Bth = 3-benzothienyalanine
Bip = 4,4'-biphenylalanine
Tic = tetrahydroisoquinoline-3-carboxylic acid
Aib = aminoisobutyric acid
Anb = α-aminonormalbutyric acid
Dip = 2,2-diphenylalanine

The compounds of the present invention can be provided in the form of pharmaceutically acceptable salts. Examples of preferred salts are those with therapeutically acceptable organic acids, e.g., acetic, lactic, maleic, citric, malic, ascorbic, succinic, benzoic, salicylic, methane sulfonic, toluene sulfonic, trifluoroacetic, or pamoic acid, as well as polymeric acids such as tannic acid or carboxymethyl cellulose, and salts with inorganic acids, such as the hydrohalic acids, e.g., hydrochloric acid, sulfuric acid, or phosphoric acid and the like.

Analysis

The structure-activity relationships of amylin and amylin analogs were studied both in an in vitro model using a mouse muscle cell line, $C_2C_{12}$, and an in vivo model using Sprague Dawley rats.

In the in vitro studies, insulin stimulated the glucose uptake by the $C_2C_{12}$ cell line in a dose-dependent manner and this was attenuated by rat amylin (100 pM). However, rat amylin did not exhibit any effect on the basal glucose uptake by this cell line. Cholera toxin did not have any effect on insulin stimulated glucose uptake but blocked the inhibitory effect of rat amylin.

Several partial sequences of human and rat amylin and their analogs were synthesized and their effects investigated in the in vitro and in vivo models.

Peptide Synthesis

Human and rat amylin were synthesized according to the procedures set forth in Balasubramaniam et al., Peptides, 12:919-924 (1991). The synthetic peptides were characterized by sequence and mass spectral analyses, and were found to be greater than 97% pure by analytical reversed-phase chromatography.

Peptide synthesis was accomplished on an Applied Biosystem Model 430A synthesizer. HPLC was carried out on a Waters Model 600 solvent delivery system in conjunction with a U6K injector, Model 481 spectrophotometer and Baseline 810 Data collection software in an IBM-XT computer. Protected amino acid derivatives (Peninsula, CA), synthesis reagents (Applied Biosystems, CA) and solvents (Fischer Scientific, OH) were obtained commercially and used without further purification. Paramethylbenzhydroxylamine (MBHA) resin (0.45 mmol, $NH_2$ group) was placed in the reaction vessel of the synthesizer and the amino acid derivatives were coupled automatically using the standard program provided by the manufacturers modified to incorporate a double

coupling procedure. All amino acids were coupled using 2.2 equivalents of preformed symmetrical anhydrides. Arg, Asn, and Gln, however, were coupled as preformed 1-hydroxybenzotriazole esters (4.4 equivalent) to avoid deamidations or lactam formation. At the end of the synthesis the N-α-Boc group was removed, and the peptide resin (1.3 g) was treated with hydrogen fluoride (~10/ml) containing dimethylsulfide (~0.8 ml), p-cresol (~0.8 g) and p-thiocresol (~0.2 g) for one hour at -2 to 4°C. HF was evacuated and the residue transferred to a fritted filter funnel with diethyl ether, washed repeatedly with diethyl ether, extracted with acetic acid (2X15 ml) and lyophilized. The crude peptide (100 mg) thus obtained was dissolved in 6 M guanidine HCl (6 ml), diluted with 500 ml of distilled water and the Ph adjusted to 8 with ammonia. A solution of 0.1% potassium ferricyanide (w/v) was then added gradually with constant stirring until a permanent yellow color persisted. After stirring for an additional 30 minutes, the Ph of the solution was adjusted to 5 with acetic acid. The solution was then stirred with anion-exchange resin (AG-3, $Cl^-$ form, 10 g wet weight) for 30 minutes, filtered through 0.45 microns filter and pumped into a semipreparative reversed phase column and purified as described in Balasubramaniam et al., Peptides, 12:919-924 (1991). The overall yield of rat and human amylin thus obtained varied between 10-20%.

## In Vitro Assays

$C_2C_{12}$ cells were cultured at 37°C in a humidified 5% $CO_2$ atmosphere, in low glucose (1 g/l) DMEM medium containing 20% fetal bovine serum, and 0.5% chick embryo extract (growth medium). Cells were seeded in 75-$cm^2$ flasks at a density of $1x10^6$ cells per flask. When the cells became confluent (3-4 days), they were trypsinized (0.25% trypsin) and washed with growth medium. The final

cell pellet was suspended in growth medium and seeded at a density of $2.5\text{-}10^4$ cells/well into 24 well plates (16 mm diameter) and allowed to grow to 70% confluence (3 days). To induce fusion, the mononucleated myoblasts were exposed to medium containing 10% horse serum instead of 20% FBS (fusion medium). Fusion media was changed every day to avoid the premature detachment of cells and the cells were almost completely fused into multinucleated myotubes by the 9th day (6 days in fusion medium). Medium was changed one day before the experiment.

2-deoxyglucose uptake in $C_2C_{12}$ myotubes was determined as described in Klip et al., Biochem. J., 242:131-136 (1987). In brief, cells were washed with PBS (phosphate-buffered saline) and incubated for 5h in the serum-free, high-glucose (25 mM) DMEM medium. At the end of incubation, cells were washed with PBS and different doses of amylin or amylin analogs (10pM- 10μM) were added and incubated with 2-deoxy-[$^3$H]-glucose (1mM) for 10 min. Non-carrier-mediated uptake was determined by incubating the cells with cytochalasin B (15 μM). Uptake was terminated by rapidly aspirating the solution, and cells were washed with ice-cold PBS. Cell-associated radioactivity was determined by lysing the cells in 1 M NaOH and the aliquots were neutralized and counted in a scintillation counter. Protein content of the aliquots was determined by the Lowry method.

After seeding, the undifferentiated mononucleated myoblasts grew logarithmically and reached 70% confluence by day 3. Fused cells were detected by day 5 and contained >90% multinucleated myotubes by day 9 (6 days in fusion media). In 6-day-old cells there was a 30% increase in glucose uptake in response to insulin compared to a 68-115% increase in 9-day-old cells. These results are similar to earlier observations (Klip et al.,

supra). The low insulin response by 6-day-old cells, presumably, is due to the presence of undifferentiated myoblasts with low insulin-receptor density as evident in the $L_6$ muscle cell line (Beguinot et al., Endocrinology, 18:446-455 (1986)). Because of these findings, and the observation that insulin stimulated glucose uptake in 9-day-old cells in a dose-dependent manner, we used 9-day-old $C_2C_{12}$ cells to test the effects of amylin or amylin analogs on insulin-stimulated glucose uptake. The maximal insulin-stimulated response was observed at 100 nM and remained plateaued at further increasing doses. The insulin-stimulated glucose uptake in $C_2C_{12}$ myotubes appears to occur mainly through facilitated diffusion because cytochalasin B(15 $\mu$M) inhibited >90% of insulin-stimulated 2-deoxyglucose uptake by the cells.

In Vivo Assays

Sprague Dawley rats (Zivic Miller, Zelienople, PA) used in this investigation were housed individually in air-conditioned rooms (22-24°C) under 12-hour light/dark cycle with ad lib access to Purina rat chow and water.

Sprague Dawley rats weighing about 300g were fasted overnight (18-22 hrs). Rats were then anesthetized with sodium pentobarbital (40 mg/kg) and catheters were implanted in the jugular vein. Saline (0.1 ml), rat amylin (50 $\mu$g) in saline (0.1ml) or peptide fragments/analogs (100$\mu$g) in saline (0.1 ml) were injected through the jugular vein and then flushed with another 0.1 ml of saline. In the cases of studying antagonistic effects, injection of peptide fragments/analogs (100$\mu$g) in saline (0.1 ml) were followed 2 min. later with rat amylin (50 $\mu$g) in saline (0.1 ml) injection. 30 min. after the injection of the peptides, blood (4-5 ml) was drawn through the jugular vein and collected in heparinized tubes containing aprotinin (10 $\mu$l). Plasma was obtained by

centrifugation. Plasma glucose and insulin levels were determined by the glucose oxidase method (Model 27 glucose analyzer, Yellow Springs Instruments, Yellow Springs, OH) and a radioimmunoassay kit (Peninsula Laboratories, Belmont, CA), respectively.

Results

Referring to Fig. 2, one of the antagonists of the invention, N-α-ac-human amylin(8-23)-$NH_2$, exhibited no significant effect on insulin stimulated glucose uptake in the in vitro assay when tested separately. Still referring to Fig. 2, the presence of N-α-ac-human amylin (8-23)-$NH_2$ (1μM) with human amylin consistently shifted the inhibitory dose-response curve of human amylin on insulin stimulated glucose uptake to the right (i.e., higher concentrations of human amylin), increasing the $IC_{50}$ value from 0.20 nM to 350 nM.

In vivo effects of N-α-ac-human amylin (8-23)-$NH_2$ were investigated in anesthetized (45 mg/kg) Sprague Dawley rats (~300 g) fasted overnight (≥ 20 h). The following samples were injected via a cannulated jugular vein into individual rats: (1) 100 μl of saline (n = 5), (2) rat amylin (50μg), (3) N-α-ac-human amylin (8-23)-$NH_2$ (100 μg), and (4) N-α-ac-human amylin (8-23)-$NH_2$ (100μg) followed 2 min later by rat amylin (50μg). Thirty minutes after injection, 4-5 ml blood was collected in heparinized tubes from each of the rats and the plasma separated by centrifugation. Plasma glucose and insulin levels were subsequently determined, and the results are set forth in Fig. 3a and 3b, respectively.

Referring to Fig. 3a, rat amylin significantly increased the plasma glucose level compared to the saline control, while N-α-ac-human amylin (8-23)-$NH_2$ significantly decreased the plasma glucose levels relative to the control, probably by antagonizing the

effects of endogenous amylin. Still referring to Fig. 3a, N-α-ac-human amylin (8-23)-$NH_2$ significantly attenuated the elevation of plasma glucose by rat amylin in the rat which received both N-α-ac-human amylin (8-23)-$NH_2$ and rat amylin (i.e. plasma glucose levels were brought down near the control value). The p values in Fig. 3a and 3b, and throughout, refer to values obtained using the ANOVA program with n equal to 5 to 8.

These observations confirm that N-α-ac-human amylin (8-23)-$NH_2$ is a potent antagonist of human amylin in vitro, and of rat amylin in vivo.

Referring to Fig. 4, human amylin (1-23)-$NH_2$ inhibited insulin stimulated glucose uptake in the in vitro assay in a manner similar to human amylin. Still referring to Fig. 4, human amylin (1-23)-$NH_2$ exhibited a dose-response inhibitory effect on insulin-stimulated glucose uptake by $C_2C_{12}$ cells with a potency comparable to that of intact human amylin.

Referring to Fig. 5a, human amylin (1-23)-$NH_2$ attenuated rat amylin induced hyperglycemia.

Referring to Fig. 6, [$Anb^{2,7}$] rat amylin(1-23)-$NH_2$ inhibited the insulin stimulated glucose uptake in the in vitro assay in a manner qualitatively similar to rat amylin(1-23)-$NH_2$. Referring to Fig. 6 and Fig. 4, rat amylin (1-23)-$NH_2$ exhibited a dose-response inhibitory effect on insulin-stimulated glucose uptake by $C_2C_{12}$ cells with a potency comparable to that of intact human amylin. Still referring to Fig. 6 and Fig. 4, [$Anb^{2,7}$] rat amylin (1-23)-$NH_2$ also exhibited a potency comparable to that of human amylin.

Referring to Fig. 7, [$Anb^{2,7}$] rat amylin(1-23)-$NH_2$ had no significant effect on amylin induced hyperglycemia, but the tendency was in the direction of attenuation.

The results obtained together with reported data in the literature are set forth in Table 1 below.

| PEPTIDES | $C_2C_{12}$-effect on insulin stimulated glucose uptake[1] | ANESTHETIZED RATS PLASMA GLUCOSE[1,2] | plasma $Ca^{2+}$ |
|---|---|---|---|
| 1. human amylin ("HA") | inhibits (Fig. 2) | elevates[3] | lowered[4] |
| 2. rat amylin ("RA") | inhibits[5] | elevates[3] | lowered[4] |
| 3. HA(1-23)-$NH_2$ | inhibits (similar to human amylin) (Fig. 4) | 1. lowers basal (Fig. 5a)<br>2. attenuates amylin induced hyperglycemia | N.D. |
| 4. RA(1-23)-$NH_2$ | inhibits (similar to human amylin) (Fig. 6) | N.D. | N.D. |
| 5. [Anb $^{2,7}$]\|RA(1-23)-$NH_2$ | inhibits (similar to human amylin) (Fig. 6) | 1. lowers basal (Fig. 7a)<br>2. no effect on amylin induced hyperglycemia | N.D. |
| 6. N-α-Ac-HA((8-23)-$NH_2$ | 1. no effect<br>2. attenuates amylin effects (Fig. 2) | 1. lowers basal (Fig. 3a)<br>2. attenuates amylin induced hyperglycemia | N.D. |

1. Present study; 2. effects of 100 μg peptide analogs on basal or 50 μg rat amylin induced hyperglycemia; 3. Molina et al., Diabetes, 39:260-265 (1990); and Young et al., Am. J. Physiology, 259: E457-461 (1990); 4. Data et al., Biochem. Biophys. Res. Commun., 162:876-881 (1989); 5. Sheriff et al., Biochim. Biophys. Acta, 1136: 219-222 (1992).

The agonist or antagonist effect of other amylin analogs of the invention may be determined by the assays described above.

USE

Amylin inhibits insulin stimulated glucose uptake and glycogen synthesis, and increases the hepatic glucose output. Therefore, it appears that a particular ratio of insulin to amylin is required to maintain the normal plasma glucose levels.

The amylin agonists and antagonists of the invention have useful applications in treating Type I and II diabetes mellitus, respectively. Since humans with Type II diabetis mellitus have elevated levels of amylin and elevated blood glucose levels, administration of an amylin antagonist of the invention in an amount sufficient to decrease blood glucose levels to normal or clinically acceptable levels provides therapeutic results. Humans with Type I diabetis mellitus have decreased levels of both insulin and amylin, and when treated with insulin have a tendency to develop hypoglycemia. Administration of an amylin agonist of the invention in an amount sufficient to increase blood glucose levels to normal or clinically acceptable levels in response to insulin induced hypoglycemia, together with a therapeutic amount of insulin, provides therapeutic results.

Amylin agonists of the invention decrease serum calcuim levels and may be administered to humans to treat hypercalcemia. Amylin agonists of the invention exhibit an appetite suppressant effect, while amylin antagonists increase appetite. Amylin agonists and antagonists of the invention are therefore useful in controlling food intake. For example, amylin agonists of the invention may be administered for the treatment of obesity.

The peptides of the invention may be administered to a human in one of the traditional modes (e.g., orally, parenterally, transdermally, or transmucosally), or in a sustained release formulation using a biodegradable biocompatible polymer.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: A. Balasubramaniam

(ii) TITLE OF INVENTION: AMYLIN ANTAGONISTS AND AGONISTS

(iii) NUMBER OF SEQUENCES: 7

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: Fish & Richardson
(B) STREET: 225 Franklin Street
(C) CITY: Boston
(D) STATE: Massachusetts
(E) COUNTRY: U.S.A.
(F) ZIP: 02110-2804

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: 3.5" Diskette, 1.44 Mb
(B) COMPUTER: IBM PS/2 Model 50Z or 55SX
(C) OPERATING SYSTEM: MS-DOS (Version 5.0)
(D) SOFTWARE: WordPerfect (Version 5.1)

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: 08/060,265
(B) FILING DATE: 12 May 1993

(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: Clark, Paul T.
(B) REGISTRATION NUMBER: 30,162
(C) REFERENCE/DOCKET NUMBER: 00537/078WO1

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(B) TELEFAX: (617) 542-8906
(C) TELEX: 200154

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 16
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

N $\alpha$ Ac Ala Thr Gln Arg Leu Ala Asn Phe Leu Val His Ser Ser Asn Asn Phe $NH_2$
5 10 15

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 16
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

N α Ac Ala Thr Gln Arg Leu Ala Asn Phe Leu Val Arg Ser Ser Asn Asn Leu $NH_2$
5 10 15

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 23
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

Lys Cys Asn Thr Ala Thr Cys Ala Thr Gln Arg Leu Ala Asn Phe Leu
5 10 15
Val His Ser Ser Asn Asn Phe $NH_2$
20

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 23
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

Lys Cys Asn Thr Ala Thr Cys Ala Thr Gln Arg Leu Ala Asn Phe Leu
5 10 15
Val Arg Ser Ser Asn Asn Leu $NH_2$
20

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 23
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

Lys Anb Asn Thr Ala Thr Anb Ala Thr Gln Arg Leu Ala Asn Phe Leu
5 10 15
Val Arg Ser Ser Asn Asn Leu $NH_2$
20

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 37
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

```
Lys Cys Asn Thr Ala Thr Cys Ala Thr Gln Arg Leu Ala Asn Phe Leu
                5                  10                  15
Val His Ser Ser Asn Asn Phe Gly Ala Ile Leu Ser Ser Thr Asn Val
            20                  25                  30
Gly Ser Asn Thr Tyr
        35
```

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 37
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

```
Lys Cys Asn Thr Ala Thr Cys Ala Thr Gln Arg Leu Ala Asn Phe Leu
                5                  10                  15
Val Arg Ser Ser Asn Asn Leu Gly Pro Val Leu Pro Pro Thr Asn Val
            20                  25                  30
Gly Ser Asn Thr Tyr
        35
```

What is claimed is:

1. An amylin analog of the amino acid formula:

$R_1$
\
$R_2$-X-$A^8$-$A^9$-$A^{10}$-$A^{11}$-$A^{12}$-$A^{13}$-$A^{14}$-$A^{15}$-$A^{16}$-$A^{17}$-$A^{18}$-$A^{19}$-A-$^{20}$-$A^{21}$-

$A^{22}$-$A^{23}$-Y-Z

wherein:

X is a chain of 0-5 amino acids, inclusive, the N-terminal one of which is bonded to $R_1$ and $R_2$;

Y is a chain of 0-4 amino acids, inclusive, the C-terminal one of which is bonded to Z;

Each of $R_1$, and $R_2$, independently, is H, $C_1$-$C_{12}$ alkyl (e.g., methyl), $C_6$-$C_{18}$ aryl (e.g., phenyl, naphthaleneacetyl), $C_1$-$C_{12}$ acyl (e.g., formyl, acetyl, and myristoyl), $C_7$-$C_{18}$ aralkyl (e.g., benzyl), or $C_7$-$C_{18}$ alkaryl (e.g., *p*-methylphenyl);

$A^8$ is Ala, Nal, Thi, Phe, Bth, Pcp, N-Me-Thr, Aib, or Anb;

$A^9$ is Thr, Ala, Anb, Aib, Ser, N-Me-Ser, or N-Me-Thr;

$A^{10}$ is Gln, Ala, Asn, N-Me-Gln, Gly, Nva, Aib, or Anb;

$A^{11}$ is Arg, homo-Arg, diethyl-homo-Arg, Lys-$\epsilon$-NH-R (where R is H, a branched or straight chain $C_1$-$C_{10}$ alkyl group, or an aryl group), Orn, or Lys;

$A^{12}$ is Leu, Ile, Val, Aib, Anb, or N-Me-Leu;

$A^{13}$ is Ala, Nal, Thi, Phe, Bth, Pcp, N-Me-Thr, Aib, or Anb;

$A^{14}$ is Asn, Ala, Gln, Gly, N-Me-Asn, Nva, Aib, or Anb;

$A^{15}$ is Phe, or any aromatic amino acid with or without substituents;

$A^{16}$ is Leu, Ile, Val, Aib, Anb, or N-Me-Leu;

$A^{17}$ is Val, Ile, Aib, Anb, or N-Me-Val;

$A^{18}$ is His, Thr, 3-Me-His, 1-Me-His, β-pyrozolylalanine, N-Me-His, Arg, homo-Arg, diethyl-homo-Arg, Lys-ε-NH-R (where R is H, a branched or straight chain $C_1$-$C_{10}$ alkyl group, or an aryl group), Ala, Aib, Anb, or Orn;

$A^{19}$ is Ser, Thr, N-Me-Ser, N-Me-Thr, Aib, Anb, or Ala;

$A^{20}$ is Ser, Thr, N-Me-Ser, N-Me-Thr, Aib, Anb, or Ala;

$A^{21}$ is Asn, Ala, Gln, Gly, N-Me-Asn, Aib, Anb, or Nva;

$A^{22}$ is Asn, Ala, Gln, Gly, N-Me-Asn, Aib, Anb, or Nva;

$A^{23}$ is Phe, any aromatic amino acid with or without substituents, Leu, Ile, Val, Aib, Anb, Ala, or N-Me-Leu; and

Z is $NHR_3$ or $OR_3$; wherein $R_3$ is H, $C_1$-$C_{12}$ alkyl, $C_7$-$C_{10}$ phenylalkyl, $C_3$-$C_{20}$ alkenyl, $C_3$-$C_{20}$ alkinyl, phenyl, or naphthyl.

or a pharmaceutically acceptable salt thereof.

2. An amylin analog of claim 1 which is an antagonist.

3. An amylin analog of claim 2 corresponding to the N-α-acetyl derivative of amino acids 8 through 23 of human amylin with an amidated carboxy at the C-terminus ("N-α-ac-human amylin (8-23)-$NH_2$") having the formula: N-α-ac-Ala-Thr-Gln-Arg-Leu-Ala-Asn-Phe-Leu-Val-His-Ser-Ser-Asn-Asn-Phe-$NH_2$, or a pharmaceutically acceptable salt thereof.

4. An amylin analog of claim 2 having the amino acid formula:

N-α-Ac-Ala-Thr-Gln-Arg-Leu-Ala-Asn-Phe-Leu-Val-Arg-Ser-Ser-Asn-Asn-Leu-$NH_2$, or a pharmaceutically acceptable salt thereof.

5. An amylin analog of the amino acid formula:

$$R_2(R_1)-X-A^1-A^2-A^3-A^4-A^5-A^6-A^7-A^8-A^9-A^{10}-A^{11}-A^{12}-A^{13}-A^{14}-A^{15}-A^{16}-A^{17}-A^{18}-A^{19}-A^{20}-A^{21}-A^{22}-A^{23}-Y-Z$$

wherein

X is a chain of 0-5 amino acids, inclusive, the N-terminal one of which is bonded to $R_1$ and $R_2$;

Y is a chain of 0-4 amino acids, inclusive, the C-terminal one of which is bonded to Z;

Each of $R_1$, and $R_2$, independently, is H, $C_1$-$C_{12}$ alkyl, $C_6$-$C_{18}$ aryl, $C_1$-$C_{12}$ alyl, $C_7$-$C_{18}$ aralkyl, or $C_7$-$C_{18}$ alkaryl;

$A^1$ is Lys, Arg, homo-Arg, diethyl-homo-Arg, Lys-ε-NH-R (where R is H, a branched or straight chain $C_1$-$C_{10}$ alkyl group, or an aryl group), or Orn;

$A^2$ is Cys, or Anb;

$A^3$ is Asn, Ala, Gln, Gly, N-Me-Asn, Aib, Anb, or Nva;

$A^4$ is Thr, Ser, N-Me-Ser, N-Me-Thr, Ala, Aib, or Anb;

$A^5$ is Ala, Nal, Thi, Phe, Bth, Pcp, N-Me-Ala, Aib, or Anb;

$A^6$ is Thr, Ser, N-Me-Ser, or N-Me-Thr, Ala, Aib, or Anb;

$A^7$ is Cys, or Anb;

$A^8$ is Ala, Nal, Thi, Phe, Bth, Pcp, N-Me-Ala, Aib, or Anb;

$A^9$ is Thr, Ser, N-Me-Ser, N-Me-Thr, Ala, Aib, or Anb;

$A^{10}$ is Gln, Ala, Asn, N-Me-Gln, Gly, Nva, Aib, or Anb;

$A^{11}$ is Arg, homo-Arg, diethyl-homo-Arg, Lys-$\epsilon$-NH-R (where R is H, a branched or straight chain $C_1$-$C_{10}$ alkyl group, or an aryl group), or Orn;

$A^{12}$ is Leu, Ile, Val, Aib, Anb, or N-Me-Leu;

$A^{13}$ is Ala, Nal, Thi, Phe, Bth, Pcp, N-Me-Ala, Aib, or Anb;

$A^{14}$ is Asn, Ala, Gln, Gly, N-Me-Asn, Aib, Anb, or Nva;

$A^{15}$ is Phe, or any aromatic amino acid with or without substituents;

$A^{16}$ is Leu, Ile, Val, Aib, Anb, or N-Me-Leu;

$A^{17}$ is Val, Ile, Aib, Anb, or N-Me-Val;

$A^{18}$ is His, Thr, 3-Me-His, 1-Me-His, $\beta$-pyrozolylalanine, N-Me-His, Arg, homo-Arg, diethyl-homo-Arg, Lys-$\epsilon$-NH-R (where R is H, a branched or straight chain $C_1$-$C_{10}$ alkyl group, or an aryl group), Orn, Ala, Aib, or Anb;

$A^{19}$ is Ser, Thr, N-Me-Ser, N-Me-Thr, Ala, Aib, or Anb;

$A^{20}$ is Ser, Thr, N-Me-Ser, N-Me-Thr, Ala, Aib, or Anb;

$A^{21}$ is Asn, Ala, Gln, Gly, N-Me-Asn, Aib, Anb, or Nva;

$A^{22}$ is Asn, Ala, Gln, Gly, N-Me-Asn, Aib, Anb, or Nva;

$A^{23}$ is Phe, any aromatic amino acid with or without substitutions, Leu, Ile, Val, Aib, Anb, Ala, or N-Me-Leu; and

Z is $NHR_3$ or $OR_3$; wherein $R_3$ is H, $C_1$-$C_{12}$ alkyl, $C_7$-$C_{10}$ phenylalkyl, $C_3$-$C_{20}$ alkenyl, $C_3$-$C_{20}$ alkinyl, phenyl, or naphthyl.

or a pharmaceutically acceptable salt thereof.

6. An amylin analog of claim 5 corresponding to amino acids 1 through 23 of human amylin with an amidated carboxy at the C-terminus ("human amylin (1-23)-$NH_2$") having the formula:

Lys-Cys-Asn-Thr-Ala-Thr-Cys-Ala-Thr-Gln-Arg-Leu-Ala-Asn-Phe-Leu-Val-His-Ser-Ser-Asn-Asn-Phe-$NH_2$, or a pharmaceutically acceptable salt thereof.

7. An amylin analog of claim 5 corresponding to amino acids 1 through 23 of rat amylin, with an amidated carboxy at the C-terminus ("rat amylin (1-23)-$NH_2$") having the formula:

Lys-Cys-Asn-Thr-Ala-Thr-Cys-Ala-Thr-Gln-Arg-Leu-Ala-Asn-Phe-Leu-Val-Arg-Ser-Ser-Asn-Asn-Leu-$NH_2$, or a pharmaceutically acceptable salt thereof.

8. An amylin analog of claim 5 corresponding to the derivative of amino acids 1 through 23 of rat amylin with α-amino normal butyric acid substitutions at positions 2 and 7, and an amidated carboxy at the C-terminus ("[$Anb^{2,7}$] rat amylin (1-23)-$NH_2$") having the formula:

Lys-Anb-Asn-Thr-Ala-Thr-Anb-Ala-Thr-Gln-Arg-Leu-Ala-Asn-Phe-Leu-Val-Arg-Ser-Ser-Asn-Asn-Leu-$NH_2$, or a pharmaceutically acceptable salt thereof.

9. A method of treating Type II diabetes mellitus in a human being comprising administering to said human being a therapeutic amount of an amylin antagonist of claim 2.

10. The method of claim 9 in which said amylin antagonist is N-α-ac-human amylin (8-23)-$NH_2$.

11. A method of treating Type I diabetes mellitus in a human being comprising administering to said human being a therapeutic amount of an amylin analog of claim 5 which is an agonist, and a therapeutic amount of insulin.

12. A method of treating hypercalcemia in a human being comprising administering to said human being a therapeutic amount of an amylin analog of claim 5 which is an agonist.

13. A method of controlling food intake in a human being comprising administering to said human being a therapeutic amount of an amylin analog of claim 1 or claim 5.

```
1               5                  10                  15
Lys Cys Asn Thr Ala Thr Cys Ala Thr Gln Arg Leu Ala Asn Phe

16              20                  25                  30
Leu Val His Ser Ser Asn Asn Phe Gly Ala Ile Leu Ser Ser Thr

31              35
Asn Val Gly Ser Asn Thr Tyr
```

**hAMYLIN** (SEQ ID NO:6)

```
1               5                  10                  15
Lys Cys Asn Thr Ala Thr Cys Ala Thr Gln Arg Leu Ala Asn Phe

16              20                  25                  30
Leu Val Arg Ser Ser Asn Asn Leu Gly Pro Val Leu Pro Pro Thr

31              35
Asn Val Gly Ser Asn Thr Tyr
```

**rAMYLIN** (SEQ ID NO:7)

FIG. 1

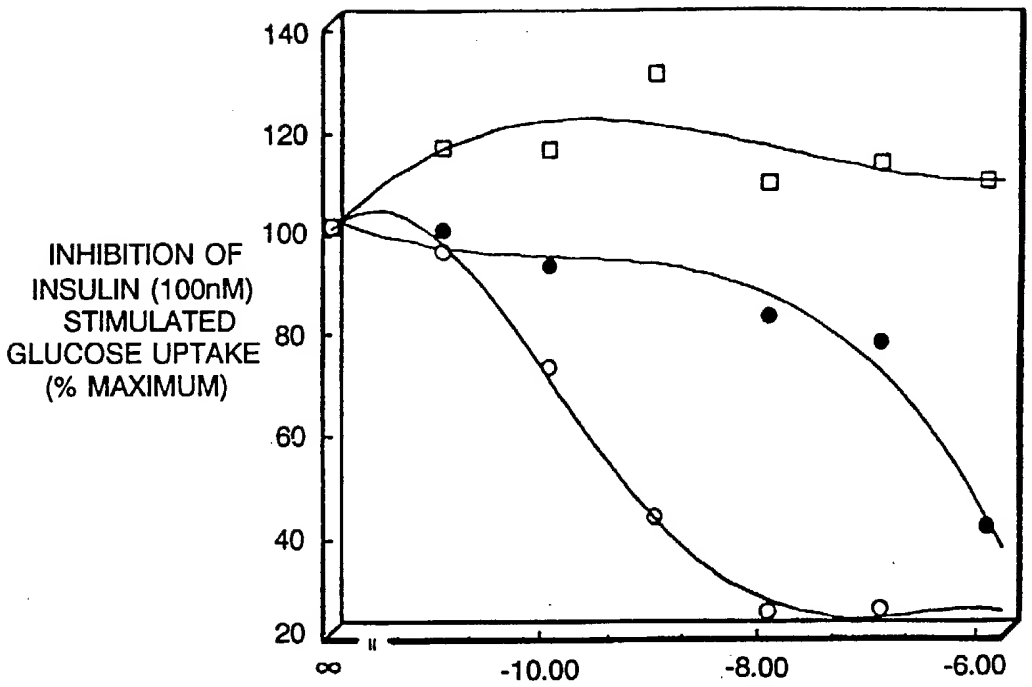

INHIBITION OF INSULIN (100nM) STIMULATED GLUCOSE UPTAKE (% MAXIMUM)
140
120
100
80
60
40
20
∞
-10.00
-8.00
-6.00
PEPTIDE, LOG [M]

FIG. 2

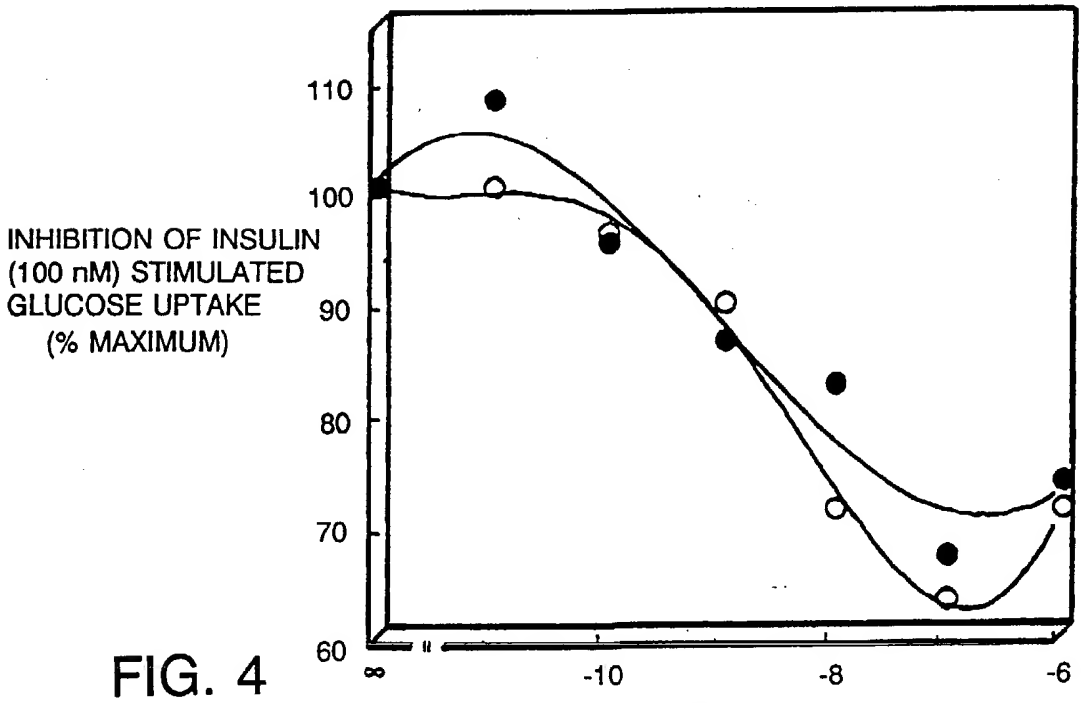

INHIBITION OF INSULIN (100 nM) STIMULATED GLUCOSE UPTAKE (% MAXIMUM)
110
100
90
80
70
60
∞
-10
-8
-6
PEPTIDES, LOG[M]

FIG. 4

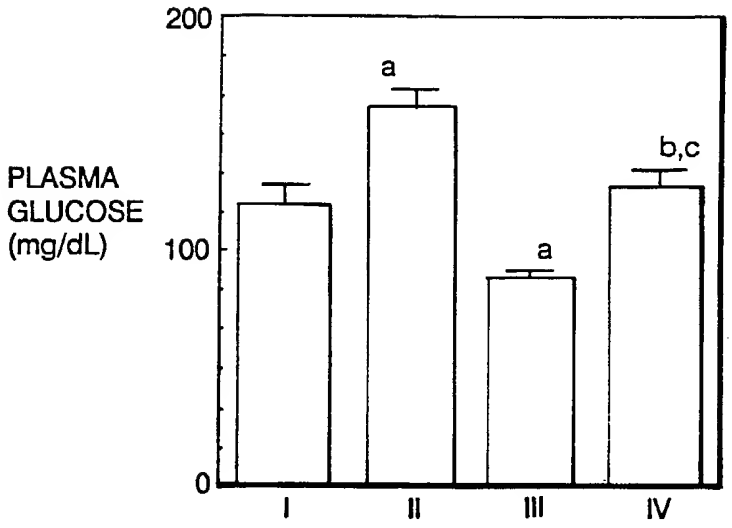
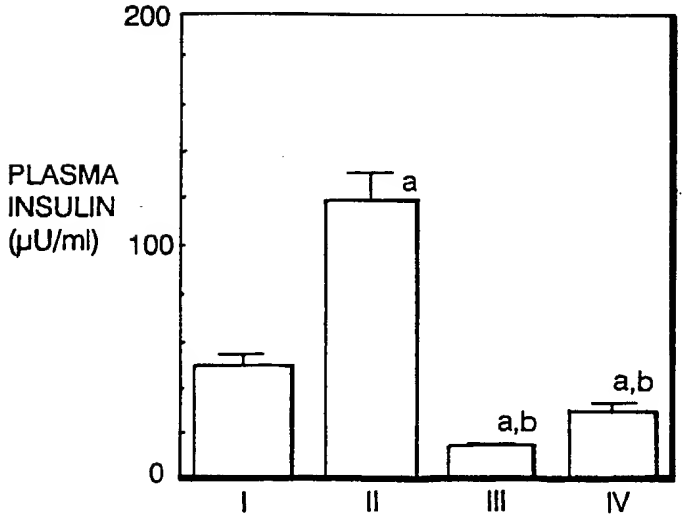



FIG. 3A

a= p< 0.05 vs control
b= not significant vs control
c= p< 0.05 vs rat amylin




I= Saline
II= Rat amylin (50μg)
III= N-α-ac-human amylin (8-23)-nh2 (100μg)
IV= N-α-ac-human amylin (8-23)-$NH^2$ (100μg) plus rat amylin (50μg)

FIG. 3B

a= p< 0.05 vs control
b= p< 0.05 vs rat amylin

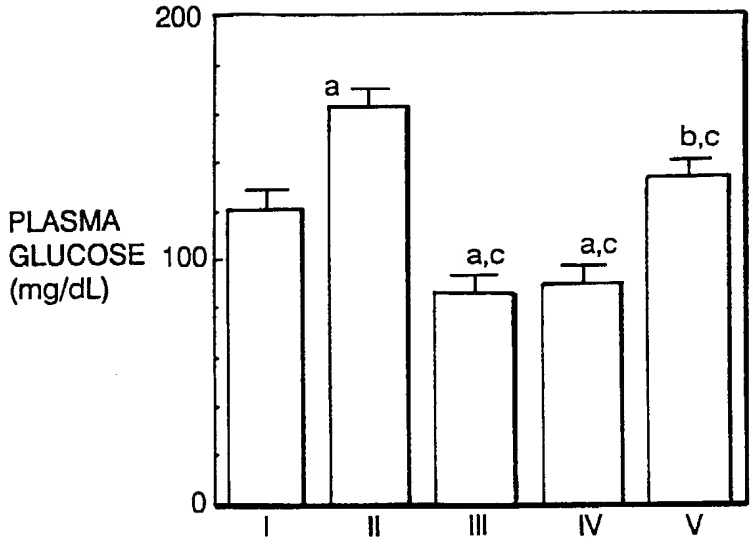



FIG. 5A

a= p< 0.05 vs control
b= not significant vs control
c= p< 0.05 vs rat amylin

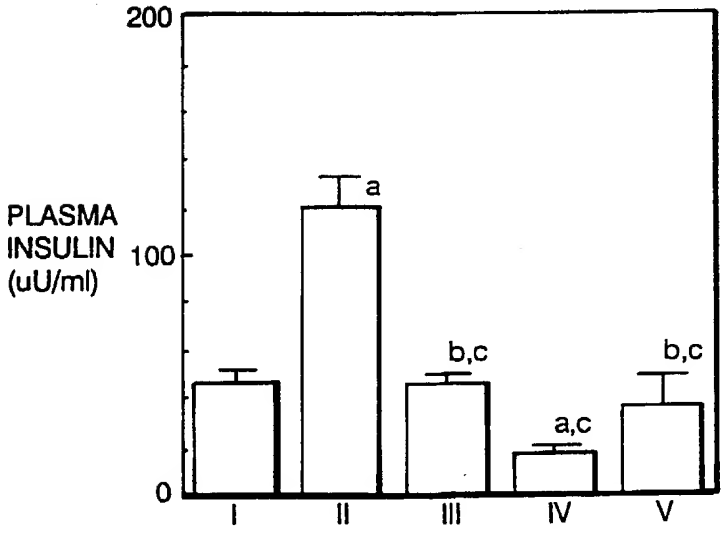



FIG. 5B

a= p< 0.05 vs control
b= not significant vs control
c= p< 0.05 vs rat amylin

I= Saline
II= Rat amylin (50µg)
III= Human amylin (1-23)-$NH^2$ (50µg)
IV= Human amylin (1-23)-$NH^2$ (100µg)
V= Human amylin (1-23)-$NH^2$ (100µg) plus rat amylin (50µg)

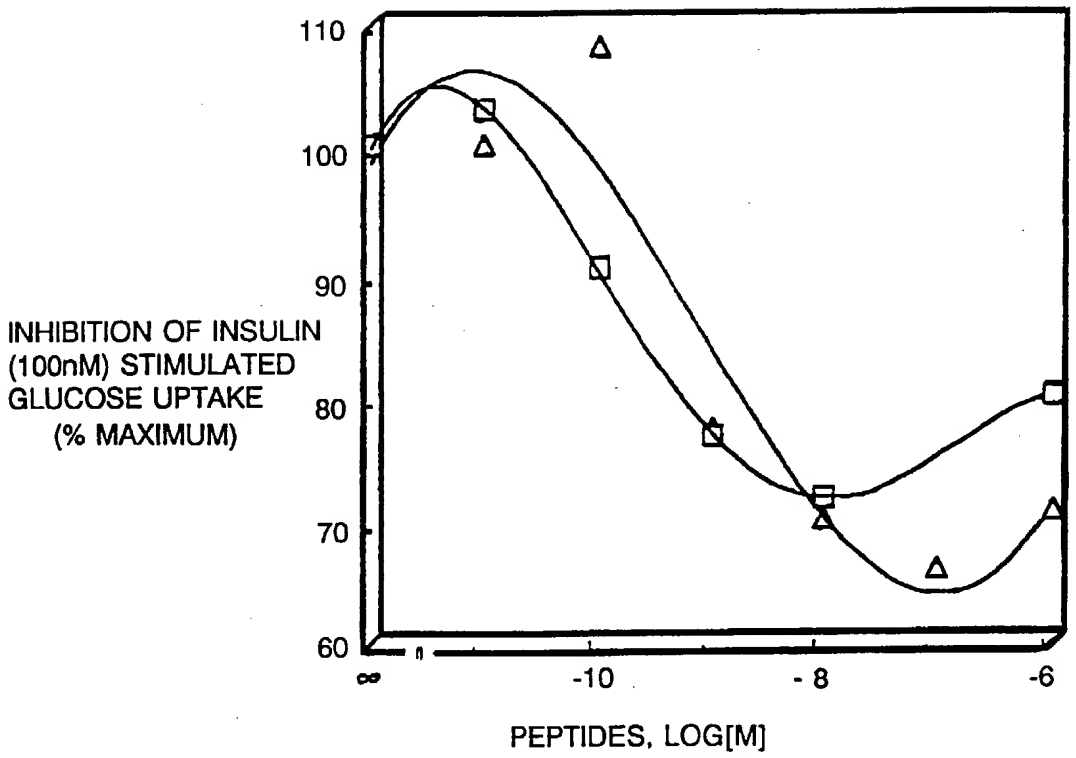
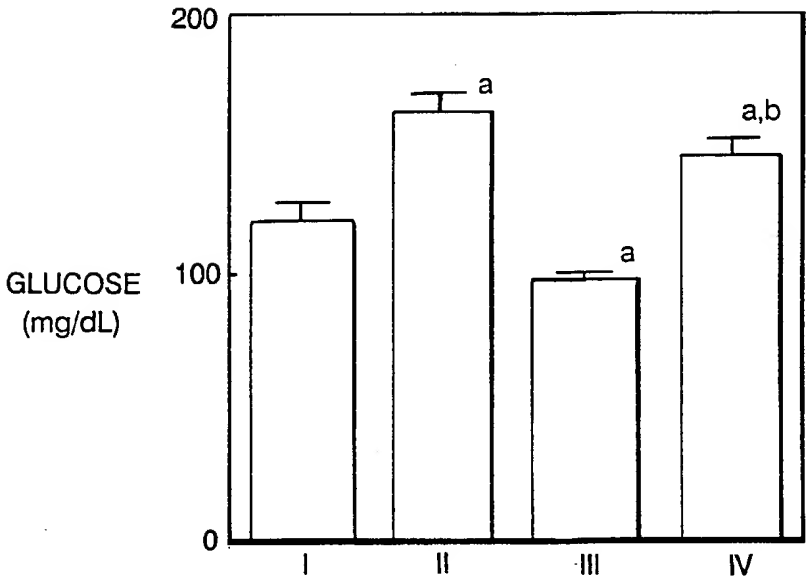

110
100
90
80
70
60
INHIBITION OF INSULIN
(100nM) STIMULATED
GLUCOSE UPTAKE
(% MAXIMUM)
∞
-10
- 8
-6
PEPTIDES, LOG[M]

FIG. 6




a= p<0.5 vs control
b= not significant vs rat amylin

FIG. 7A

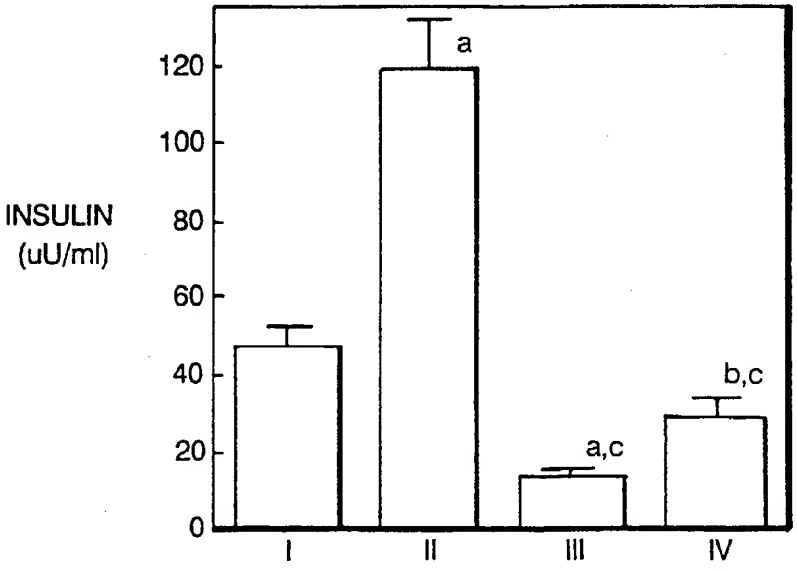



FIG. 7B

a= p< 0.05 vs control
b= not significant vs control
c=p< 0.05 vs rat amylin

I= Saline
II= Rat amylin (50µg)
III= [Anb $^{2,7}$] rat amylin (1-23)-$NH^2$ (100µg)
IV= [$Anb^{2,7}$] rat amylin (1-23)-$NH^2$ (100µg) plus Rat amylin (50µg)

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/05282

**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(5) :A61K 37/02; C07K 7/06, 7/08
US CL :530/326; 514/13
According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/326; 514/13

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAS, STN

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| A | Proceedings of the National Academy of Science USA, Vol. 85, issued 1988, Cooper et al , "Amylin found in amyloid deposits in Human Type 2 Diabetes Mellitus may be Hormone that Regulates Glycogen Metabolism in Skeletal Muscle", pages 7763-7766, see entire document. | 1-13 |
| A | Proceedings of the National Academy of Science USA, Vol. 84, issued June 1987, Westermark et al, "Amyloid Fibrils in Human Insulinoma and Islets of Langerhans of the Diabetic Cat Are Derived From A Neuropeptid-like Protein Also Present in Normal Islet Cells", pages 3881-3885, see entire document. | 1-13 |

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

| | | | |
|---|---|---|---|
| * | Special categories of cited documents: | "T" | later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention |
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| "O" | document referring to an oral disclosure, use, exhibition or other means | | |
| "P" | document published prior to the international filing date but later than the priority date claimed | | |

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 01 AUGUST 1994 | 15 AUG 1994 |
| Name and mailing address of the ISA/US<br>Commissioner of Patents and Trademarks<br>Box PCT<br>Washington, D.C. 20231<br>Facsimile No. (703) 305-3230 | Authorized officer<br>S.G. Marshall Jill Warden for<br>Telephone No. (703) 308-0196 |

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# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/05282

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| A | Biochemical and Biophysical Research Communications, Vol. 160, No. 2, issued 28 April 1989, Ohsawa et al, "Islet Amyloid Polypeptide Inhibits Glucose-Stimulated Insulin Secretion From Isolated Rat Pancreatic Islets", pages 961-967. see entire document. | 1-13 |
| A,P | US, A, 5,266,561 (COOPER ET AL) 30 November 1993, see entire document. | 1-13 |